Recurrent bacteremia with *Streptococcus dysgalactiae:* a case-control study

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Abstract

Beta-hemolytic streptococci of groups C and G, designated as *Streptococcus dysgalactiae* (SD), can cause severe and recurring invasive infections. In this case-control study, we aimed to identify clinical and molecular risk factors for recurrence of SD bacteremia. Twenty-two cases of recurrent SD bacteremia were identified and median time between episodes was six months. The most frequent clinical manifestation was skin- and soft-tissue infection. Cases and 92 controls, with single episode SD bacteremia, showed similar demographics, had similar Charlson comorbidity scores, and had similar clinical presentations. Thirty days fatality was 13 % among controls whereas none of 22 cases died. In 19 (86%) cases, the same *emm* type was encountered in both episodes. SD isolates from recurrent episodes and from single episodes had a similar *emm* type distribution. Thus, we did not identify clinical risk factors for recurrences. The high proportion of identical *emm* types in recurrent episodes indicates a host-specific colonization.

Keywords: bacteremia, beta-hemolytic streptococci, recurrent bacteremia, *Streptococcus dysgalactiae*, *emm*-type.

1. Introduction

Human pathogenic beta-hemolytic streptococci of groups C and G (GCS and GGS) have become increasingly recognized as important causes of severe infection [1-3]. The vast majority of GGS and GCS causing human infections belong to *Streptococcus dysgalactiae* (SD) and cause a spectrum of disease similar to that of *Streptococcus pyogenes* [3-6]. SD is further divided into *subsp. equisimilis* which is beta-hemolytic and can have different Lancefield groups and *subsp. dysgalactiae* which is not beta-hemolytic and display Lancefield group C [7]. Otherwise, the two subspecies are not easily separated by standard methods such as biochemistry or sequencing of the 16S rRNA gene [7,8]. Among GCS causing human infections, some isolates are *Streptococcs equi* [5,9], and some isolates among GGS are *Streptococcus canis* [10]. Typing of SD is commonly performed through sequencing of the *emm* gene encoding the M protein. The most common focus of invasive SD infection is skin- and soft tissue, followed by bacteremia of unknown origin, but also septic arthritis, and infective endocarditis occurs [3]. Invasive infections are frequently seen in elderly patient and those with underlying medical conditions [5,11]. The mortality rates in SD bacteremia have been reported to be between 8 and 15 % [5,11,12]. SD bacteremia has a tendency to recur and rates of recurrence between 3 and 9 % have been reported [6,13-16]. In such reports, up to four recurrences were described with a median interval of 8 months (range 1-64 months) between the episodes [3,6,15,17]. There has been some speculation whether or not recurrence is associated with a specific *emm* SD type or- clinically- with specific underlying conditions. In previous case series, recurrence was caused by the same *emm* type SD in 7 of 8, 3 of 4, and in 2 of 5 patients and isolates from a given patient displaying the same *emm* type were highly genetically related [3,6,15]. The SD *emm* type causing

recurrence has varied between reports but StG485.0 [6,17] and StG6 [3] have been isolated from many cases. These studies were underpowered to detect if particular SD types are more prone to cause recurrent bacteremia. Studies looking at the clinical associations reported the presence of genital cancer (particularly cervical cancer), a history of cellulitis [6], or "chronic lymphatic abnormalities" [15] more frequent in cases with recurrent SD bacteremia. The present case-control study was performed to identify bacterial and host factors associated with increased risk for recurrence of SD bacteremia.

2. Materials and methods

Cases of recurrent bacteremia with GCS and GGS between 2003 and 2013 were identified in the databank of the department of clinical microbiology in Malmö/Lund. The laboratory is the only one serving a population of around 1.2 million inhabitants. For each case, the two preceding and the two following patients with bacteremia with SD of the same streptococcal group were selected as controls (i.e. case-control ratio 1:4). The identification of the bacteria by the diagnostic laboratory had depended on a typical appearance upon Gram-staining and on blood agar as well as on latex agglutination (Streptex, Remel, Lenexa, KS, USA). We cultured the bacteria on blood agar in 5% CO_2 at 37°C over night and directly transferred them to grids and subjected them to analysis with Ultraflextreme matrix-assisted laser desorption ionization - time of flight mass spectrometry (MALDI-TOF MS) (Bruker Daltonics, Bremen, Germany), using the Biotyper version 3.0 software. A score of above 2.0 was considered to confirm the identification to the species level. The *emm* gene encoding the SD M protein was sequenced and type was determined as described (http://www.cdc.gov/streplab).

Data on clinical presentation was gathered from the medical records of the respective patient according to a predefined questionnaire modified from [18]. Sepsis was assessed as described [19]. The study was approved by the Regional Ethics Committee in Lund (no 2013/31). Statistical analyses were performed using the Prism 6 software. If not otherwise stated, Fischer's exact test was used to compare categorical variables and Mann-Whitney-U test used for continuous variables. A p-value< 0.05 was considered significant.

3. Results

3.1 Patients with recurrence

Twenty-three patients with recurrent episodes of bacteremia with GCS or GGS were identified among a total of 593 episodes. Their features are described in table 1. In 19 patients GGS, and in 4 patients GCS was the microorganism causing recurring bacteremia. When MALDI-TOF MS species identification was used, SD was demonstrated in 22 cases, and *Streptococcus canis* (GGS) in one case. In 20 patients two episodes of bacteremia were recorded, and in three patients three episodes were identified. The median time between the first and the second episodes was six months (range 1-53 months), whereas the time interval between the second and third episode was four, six, and nineteen months respectively. In 19 patients, the recurrence was caused by the same *emm* type. In three cases, different *emm* types were noted. The *S. canis* isolate was non-typeable.

Skin- and soft-tissue infection was the most frequent clinical manifestation (17 of 22 patients). Erysipelas was the single most common entity comprising 50 %. In 77 % of the cases, the clinical presentation was identical in the first and the second episode. Treatment for the first episode was given with a beta-lactam antibiotic for a minimum

of 10 days. More SIRS criteria (median 3) were noted on the presentation of a recurrent episode when compared with the initial episode (median 2), though this difference was statistically not significant (p=0.2 with Wilcoxons matched-pairs signed rank test). There were no differences in CRP levels or the number of patients fulfilling criteria for severe sepsis on initial presentation and presentation at recurrence. No fatalities were recorded among the 23 patients with recurrence.

3.2 Comparison of recurrent and single episodes of SD bacteremia

3.2.1 Bacteriological aspects

92 control isolates (76 of GGS and 16 of GCS) were confirmed to be SD by MALDI-TOF MS. The type-distribution of the isolates where the same type caused recurrence (n=19) were similar to types of isolates (n=92) causing a single episode (Figure 1). Types StG643, StG480, and StG6 were common in both groups whereas StG485 was more common in the group with a single episode.

3.2.2 Clinical characteristics

Table 2 compares the demographic and clinical characteristics of patients with recurrent and single episode SD bacteremia. A previous history of erysipelas was more common in the group with recurrence but this difference was not statistically significant. Table 2 also compares the clinical presentations of patients with recurrent and single episode SD bacteremia. Erysipelas was the single most common entity comprising 50 % of patients who later recurred and 38 % of those with a single episode. Infection foci such as abscesses, bone and joint infections and infections related to medical procedures and foreign materials were more common in the group with a single episode. The differences were, however, not statistically significant.

Five patients (23 %) from the group with recurrent SD bacteremia had an initial presentation with severe sepsis; in comparison 30 patients (32 %) presented with severe sepsis in the group with a single SD bacteremia episode (p=0.6). Empirical treatment was initiated with a broad-spectrum beta lactam (e.g. a cephalosporin or carbapenems) in 52 % of cases and with a penicillin or cloxacillin in 34 % of cases with no significant differences between the groups. The total time for intravenous antibiotics was similar between the groups (7 and 6.5 days respectively) as was the total time of antibiotic treatment (Table 2). The length of hospital stay was non-significantly longer in the group with a single episode.

There were 12 fatalities (13 %) within 30 days of the positive blood culture in the control group whereas none of the 23 patients experiencing recurrence died within 30 days of the recurrence (p=0.1).

4. Discussion

We identified 23 patients with recurrent GCS and GGS bacteremia in 11 years underlining the observation that SD bacteremia has a tendency to recur. Our study, despite of being retrospective, is larger than previous case reports/series, has defined controls, and is population based. We did not identify factors that were significantly associated with an increased risk for recurrence, apart from the association with erysipelas in the first episode. Due to the nature of retrospective case-control studies, the conclusions are limited by the estimates (e.g. clinical assessment) noted in the patient chart and the relatively small sample size of cases. Nevertheless, the fact that almost 90% of the recurrences are caused by a pathogen revealing the same *emm* type is indicative for a specific host-pathogen colonization association. Moreover, none of

our patients had a history of intravenous drug use, suggesting an endogenous or host-specific source of recurrent bacteremia.

SD *subspecies equisimilis* is beta-hemolytic and can display different Lancefield groups as opposed to *subsp. dysgalactiae* which is not beta-hemolytic and carries a Lancefield group C antigen [7]. The SD isolates described here all displayed beta-hemolysis and a majority are Lancefield group G and thus they likely all belong to the *subspecies equisimilis* [7], but since MALDI-TOF MS cannot separate this subspecies from *subspecies dysgalactiae* we chose not to indicate subspecies [8]. The types StG6, StG480, and StG485 prevalent in this study have also been reported to be so by others [2,12,14,20]. Type StG643 comprising 17 % of our isolates was very recently shown to be common in Norway [21]. Also, looking solely at *emm*-types, there was no significant association with increased risk for recurrence. We were not able to analyze host factors (e.g. HLA types) to review a host-pathogen association as shown previously with GAS [22]. The risk factors reported previously; genital cancer [6] and "chronic lymphatic abnormalities" [15], were infrequent among our cases whereas a history of cellulitis, reported by Liao *et al.* to be associated with recurrence, was also observed frequently among our patients with recurrence.

The risk for recurrence in GCS/GGS bacteremia has been reported to be between 3 and 10 % and the majority of recurrences occur within the first year [6,13-16]. We could confirm this observation in our study. Since bacteremia with SD is associated with significant mortality and morbidity, the patients and their relatives should be informed of the risk for recurrence and informed to seek medical care immediately upon suspicion of recurrence. Intensified efforts to eliminate potential risk factors such as wounds are also warranted. The use of penicillin prophylaxis is a controversial issue and the dosing and duration of such treatment is unexplored. In

selected cases prophylaxis could be considered and we would favor the use of prophylaxis to patients after recurrence if there is a defined risk factor that cannot be eliminated. To improve the understanding of SD recurrent bacteremia, future studies should address if the patients with recurrence are constantly colonized or if they are reinfected as well as if their antibody response to SD differ from that of patients with single episodes.

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Legend to figure

Figure 1. Distribution of types among isolates where the same *emm* type caused the recurrent episode (n=19) represented with black bars and among isolates from single episodes (n=92) represented by grey bars. Other isolates include StG5820, StG245, and StG5420 (n=2), as well as single isolates of StC1400, StC36, and StC6979.

Table 1 Characteristics of patients with recurrent episodes of bacteremia with GCS/CCS

Age & gender	Underlying condition	Clinical syndrome 1st/2nd episode	Group	Type	Months to recur
90 M	CHF, RF	Erysipelas	GGS	StG480/485	13
87 F	De	UF/bursitis	GGS	nt	6
83 M	PE, PN	Erysipelas	GCS	StG652	2
71 M	DM, KT, CW	WI	GGS	StG480	3
69 F	DM, RF, CW, COPD	WI	GGS	StG10	2
62 M	DM, Ob, CW	Erysipelas	GGS	StG6792/652	21
56 F	LO, PE	Erysipelas	GGS	StG166	23
87 M	De, PC	UF/Erysipelas	GGS	StG485	4
84 F	S, CHF	Erysipelas	GGS	StG480	1
81 M	DM, CW, PD	Erysipelas/WI	GCS	StG62647	1
79 M	PE, cyt for LC	Erysipelas	GGS	StG6	1
69 F	AML, PAC	UF/PAC-infection	GGS	StG2078	11
68 F	SLE, CHF, COPD	Necrotizing cellulitis/UF	GCS	StG643	37
79 M	PH, PE	Erysipelas	GGS	StG480	39
62 F	DCM, Sa	Erysipelas	GGS	StG6	12
80 F	AVP	UF/Erysipelas	GCS	StG643	53
81 M	PC, CW	WI/WI/osteitis	GGS	StC74a	2, 6
75 M	PH	Cellulitis	GGS	StG485	8, 2
70 F	RT against AC	Myositis/UF	GGS	StG6	12
71 M	Ob, DM, UC	Erysipelas	GGS	StG6792/6/652	16, 10
60 M	DM, HL, Ps	Cellulitis/Erysipelas	GGS	StG643	9
57 M	Cyt for VC, PAC	UF/PAC-infection	GGS	StG480	2
65 M	PE, Ob, PN	Erysipelas	GGS	StG483	39

Abbreviations used are: CHF, congestive heart failure; RF, renal failure; nt, non-typable; De, dementia; UF, unknown focus; PE, previous erysipelas; PN, polyneuropathy; DM, diabetes mellitus; KT, kidney transplanted; CW, chronic wound; WI, wound infection; COPD, chronic obstructive pulmonary disease; Ob, obesitas; LO, lymphedema; PC, prostate cancer; S, stroke; PD, Parkinssons disease; cyt, cytostatic drugs; LC, lung cancer; AML, acute myeloic leukemia, PAC, port-á-cath; SLE, systemic lupus erythematosus; ICU, intensive care unit; DCM, dilated cardiomyopathy; Sa, sarcoidosis; AVP, aortic valve prosthesis; PH, prostate hyperplasia; RT, radiation therapy; AC, anal cancer; UC, urinary catheter; HL, Hodgekin lymphoma; Ps, psoriasis, VC, ventricular carcinoma.

Table 2. Comparison of recurrent and single episodes of bacteremia with SD

	Recurrent episode (n=22)	Single episode (n=92)	p for difference
Age, (years, median)	74	74	p=0.6
Gender (% male)	64	63	p=1
Charlson comorbidity index (mean, range)	2 (0-6)	1 (0-7)	p=0.2
Underlying disease			
Diabetes (%)	32	24	p=0.5
Chronic leg ulcer (%)	27	11	p=0.09
Previous radiation or lymph oedema (%)	14	9	p=0.4
Previous erysipelas	23	8	p=0.05
Clinical Manifestation(%)			
Skin and soft tissue	67	56	p=0.3
Bacteraemia without focus	23	24	p=1
Abscess	0	4	p=1
Bone and joint	0	5	p=0.6
Post operative or device related	0	8	p=0.3
Endocarditis	0	1	p=1
Severe sepsis at presentation (%)	23	32	p=0.6
Empirical treatment (%)			
Penicillin or cloxacillin	37	30	p=0.6
Cephalosporin or carbapenem	55	52	p=1
Other	5	11	p=0.7
No antibiotic	5	7	p=1
Treatment length (days, median)			
Intravenous	6.5	7	p=0.5
Total	14	14	p=1
Length of stay in the hospital (days, median)	7.5	11	p=0.2
Fatality proportion (%)	0	13	p=0.1

Statistical testing was performed with Mann-Whitney U test for continuous variables and with Fischer´s exact test for categorical variables.